

What is claimed is:

1           1. A method of diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising determining the presence of a mutation in  
4 exon 2 of an HFE nucleic acid in a biological sample from  
5 said mammal, wherein said mutation is not a C→G substitution  
6 at nucleotide 187 of SEQ ID NO:1 and wherein the presence of  
7 said mutation is indicative of said disorder or a genetic  
8 susceptibility to developing said disorder.

1           2. The method of claim 1, wherein said disorder is  
2 hemochromatosis.

1           3. The method of claim 1, wherein said nucleic acid  
2 is a DNA molecule.

1           4. The method of claim 1, wherein said nucleic acid  
2 is a RNA molecule.

1           5. The method of claim 1, wherein said mutation is  
2 a missense mutation at nucleotide 314 of SEQ ID NO:1.

1           6. The method of claim 5, wherein said mutation is  
2 314C.

1           7. The method of claim 6, wherein said mutation  
2 results in expression of mutant HFE gene product I105T.

1           8. The method of claim 1, wherein said mutation is  
2 at nucleotide 277 of SEQ ID NO:1.

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1 9. The method of claim 8, wherein said mutation is  
2 277C.

1 10. The method of claim 9, wherein said mutation  
2 results in expression of mutant HFE gene product G93R.

1 11. The method of claim 1, wherein said mutation is  
2 at nucleotide 193 of SEQ ID NO:1.

1 12. The method of claim 11, wherein said mutation  
2 is 193T.

1 13. The method of claim 12, wherein said mutation  
2 results in expression of mutant HFE gene product S65C.

1 14. The method of claim 1, wherein said biological  
2 sample is selected from the group consisting of whole blood,  
3 cord blood, serum, saliva, plasma, effusions, ascites,  
4 urine, stool, buccal tissue, liver tissue, kidney tissue,  
5 cerebrospinal fluid, skin, hair and tears.

1 15. The method of claim 14, wherein said biological  
2 sample is whole blood.

1 16. The method of claim 14, wherein said biological  
2 sample is saliva.

1 17. The method of claim 14, wherein said biological  
2 sample is hair.

1 18. The method of claim 1, wherein said mammal is a  
2 human.

1 19. The method of claim 1, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide  
3 primer which is 5' to exon 2 and a second oligonucleotide  
4 primer is 3' to exon 2.

1 20. The method of claim 1, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide  
3 primer which is 5' to nucleotide 314 of SEQ ID NO:1 and a  
4 second oligonucleotide primer which is 3' to nucleotide 314  
5 of SEQ ID NO:1.

1 21. The method of claim 1, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide  
3 primer which is 5' to nucleotide 277 of SEQ ID NO:1 and a  
4 second oligonucleotide primer which is 3' to nucleotide 277  
5 of SEQ ID NO:1.

1 22. The method of claim 1, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide  
3 primer which is 5' to nucleotide 193 of SEQ ID NO:1 and a  
4 second oligonucleotide primer which is 3' to nucleotide 193  
5 of SEQ ID NO:1.

1 23. The method of claim 20, 21, or 22, wherein said  
2 first oligonucleotide primer has a nucleotide sequence of  
3 SEQ ID NO:3 and said second oligonucleotide primer has a  
4 nucleotide sequence of SEQ ID NO:4.

1 24. The method of claim 20, 21, or 22, wherein said  
2 first oligonucleotide primer has a nucleotide sequence of  
3 SEQ ID NO:15 and said second oligonucleotide primer has a  
4 nucleotide sequence of SEQ ID NO:16.

1           25. A method of diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising determining the presence or absence of a  
4 mutation in an intron of HFE genomic DNA in a biological  
5 sample from said mammal, wherein the presence of said  
6 mutation is indicative of said disorder or a genetic  
7 susceptibility to developing said disorder.

1           26. The method of claim 25, wherein said mutation  
2 is in intron 4.

1           27. The method of claim 26, wherein said mutation  
2 is at nucleotide 6884 of SEQ ID NO:27.

1           28. The method of claim 27, wherein said mutation  
2 is 6884C.

1           29. The method of claim 25, wherein said mutation  
2 is in intron 5.

1           30. The method of claim 29, wherein said mutation  
2 is at nucleotide 7055 of SEQ ID NO:27.

1           31. The method of claim 30, wherein said mutation is  
2 7055G.

1           32. The method of claim 25, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide  
3 primer which is 5' to intron 4 and a second oligonucleotide  
4 primer which is 3' to intron 4.

1           33. The method of claim 25, further comprising  
2 amplifying said nucleic acid using a first oligonucleotide

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3 primer which is 5' to intron 5 and a second oligonucleotide  
4 primer which is 3' to intron 5.

1 34. A method of diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising determining the presence of a mutation in  
4 a HFE gene product in a biological sample from said mammal,  
5 wherein said mutation results in a decrease in an  
6 intramolecular salt bridge formation in said HFE gene  
7 product but is not amino acid substitution H63D, and wherein  
8 the presence of said mutation is indicative of said disorder  
9 or a genetic susceptibility to developing said disorder.

1 35. The method of claim 34, wherein said disorder  
2 is hemochromatosis.

1 36. The method of claim 34, wherein said mutation  
2 is between amino acids 23-113, inclusive, of SEQ ID NO:2.

1 37. The method of claim 34, wherein said mutation  
2 is between amino acids 58-68, inclusive, of SEQ ID NO:2.

1 38. The method of claim 34, wherein said mutation  
2 is between amino acids 60-65, inclusive, of SEQ ID NO:2.

1 39. The method of claim 34, wherein said mutation  
2 is amino acid substitution S65C.

1 40. The method of claim 34, wherein said mutation  
2 is between amino acids 90-100, inclusive, of SEQ ID NO:2.

1 41. The method of claim 34, wherein said mutation  
2 is between amino acids 92-97, inclusive, of SEQ ID NO:2.

1 42. The method of claim 34, wherein said mutation  
2 is amino acid substitution G93R.

1 43. The method of claim 34, wherein said mutation  
2 is at amino acid 95 of SEQ ID NO:2.

1 44. The method of claim 34, wherein said mutation  
2 is detected by immunoassay.

1 45. A method of diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising determining the presence of a mutation in  
4 a HFE gene product in a biological sample from said mammal,  
5 said mutation being located in the  $\alpha$  helix of said HFE gene  
6 product, wherein the presence of said mutation is indicative  
7 of said disorder or a genetic susceptibility to developing  
8 said disorder.

1 46. The method of claim 45, wherein said mutation  
2 is between amino acids 80-108, inclusive, of SEQ ID NO:2.

1 47. The method of claim 45, wherein said mutation  
2 is I105T.

1 48. The method of claim 45, wherein said mutation  
2 is G93R.

1 49. An isolated nucleic acid molecule encoding an  
2 HFE polypeptide comprising amino acid substitution I105T or  
3 the complement thereof.

1 50. An isolated nucleic acid molecule encoding an  
2 HFE polypeptide comprising amino acid substitution G93R or  
3 the complement thereof.

1 51. An isolated nucleic acid molecule encoding an  
2 HFE polypeptide comprising amino acid substitution S65C or  
3 the complement thereof.

1 52. A kit for detecting a nucleotide polymorphism  
2 associated with an iron disorder or a genetic susceptibility  
3 to developing said disorder in a mammal comprising the  
4 nucleic acid molecule of claims 49, 50, or 51.

1 53. A kit for the detection of the presence of a  
2 mutation in exon 2 of an HFE nucleic acid comprising a first  
3 oligonucleotide primer which is 5' to exon 2 and a second  
4 oligonucleotide primer is 3' to exon 2.

1 54. A substantially pure HFE polypeptide comprising  
2 amino acid substitution I105T.

1 55. A substantially pure HFE polypeptide comprising  
2 amino acid substitution G93R.

1 56. A substantially pure HFE polypeptide comprising  
2 amino acid substitution S65C.

1 57. A kit for diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising an antibody which preferentially binds to  
4 an epitope of a mutant HFE gene product, wherein said gene  
5 product comprises amino acid substitution I105T, G93R, or  
6 S65C.

1           58. A kit for diagnosing an iron disorder or a  
2 genetic susceptibility to developing said disorder in a  
3 mammal, comprising an antibody which preferentially binds to  
4 an epitope of a wild type HFE gene product, wherein said  
5 gene product comprises amino acid substitution I105, G93, or  
6 S65.

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